

Metagenomic analysis of plankton and bacterial communities in the seaweed culture environment of Kepulauan Seribu, Indonesia

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Abstract. Seaweed production in Indonesia has experienced a significant decline of 3.55% over the past five years, with a drastic reduction in the Kepulauan Seribu region, where output dropped from 196 tons in 2018 to 2 tons in 2022. Understanding the biological and environmental parameters that support seaweed cultivation, particularly microbial and plankton diversity, is essential for sustainable production. This study, conducted on *Kappaphycus alvarezii* (Doty) Doty ex P.C.Silva, 1996 cultivation sites across Kepulauan Seribu during the 2023 rainy seasons (April–May) and dry seasons (July–August), involved water quality assessment and biological sampling at twelve points near five key islands. Next-generation sequencing (NGS) of bacterial communities revealed that Alphaproteobacteria, particularly Rhodobacteriaceae, dominated across seasons, while zooplankton was prevalent during the rainy season, and phytoplankton during the dry season. Harmful algae and pathogenic bacteria were absent in the samples, indicating a generally safe environment for seaweed growth. Although some anthropogenic pollution was detected through elevated oil content and chlorophyll-a, overall water quality was deemed suitable for seaweed cultivation. The findings suggest that, with appropriate management to mitigate pollution, the Kepulauan Seribu region retains a strong potential for sustainable seaweed culture.

Key Words: metagenom, bacteria, plankton, Kepulauan Seribu, seaweed culture.

Introduction. Indonesia possesses substantial potential to establish itself as a leading maritime nation, leveraging its vast marine resources and strategic geographical location. As an archipelago with an extensive coastline, the country is uniquely positioned to capitalize on its maritime identity, which offers significant economic advantages (Hastuti et al 2023). Indonesia's fisheries sector, particularly aquaculture, plays a pivotal role in its economy by generating income, diversifying employment opportunities, and providing a critical source of animal protein for its population (Tran et al 2017). By 2023, aquaculture contributed 68.59% to the country's total fisheries production, highlighting its significance in national food security and economic resilience. Seaweed culture, in particular, is a major contributor to the aquaculture sector, reaching a total production of 10.7 million tons in 2023 (MMAF 2024). Furthermore, Indonesia's extensive potential for seaweed cultivation, particularly in regions like the Kepulauan Seribu archipelago north of Jakarta, highlights the country's considerable capacity for sector expansion, with implications for both local and national economic growth.

Over the past five years, national seaweed production has decreased by 3.55%, with a particularly sharp decline observed in the Kepulauan Seribu, Jakarta, where output dropped from 196 tons in 2018 to just 2 tons by 2022 (MMAF 2024). Several environmental factors, such as coastal abrasion, tidal waves, and changes in water quality including turbidity and nutrient fluctuations have been identified as major contributors to this decline (Suhendra et al 2024; Wijianto et al 2024). Additionally, plankton blooms exacerbate the issue by competing for nutrients, reducing the availability of essential resources for

seaweed growth (Zhu et al 2022). Pathogenic bacteria, including those responsible for ice-ice disease in species like *Kappaphycus alvarezii*, further diminish seaweed productivity (Aziz et al 2022). Therefore, understanding the diversity of bacterial and planktonic communities in aquaculture systems is critical for improving production efficiency. This can be achieved through both traditional observational methods and advanced molecular techniques, such as next-generation sequencing, which offer a more comprehensive assessment of microbial communities.

Next Generation Sequencing (NGS) has emerged as a widely recognized metabarcoding approach for analyzing microbial communities. This advanced technique allows for the comprehensive examination of community structures and the alpha and beta diversity of microbial taxa (Hendrayanti et al 2023). Compared to traditional methods, NGS offers more accurate identification of plankton species and bacterial taxa (Nafea et al 2023). It also facilitates the detection of a greater diversity of plankton genera and species, thus enhancing biodiversity assessments (Malashenkov et al 2021). In this study, we focus on analyzing the bacterial and plankton communities in the Kepulauan Seribu to evaluate the suitability of this region for marine aquaculture.

Material and Method

Sample collection. The study was conducted at the seaweed cultivation site of *Kappaphycus alvarezii* in the Kepulauan Seribu during the rainy season (April - May 2023) and the dry season (July - August 2023). Water quality measurements and sampling were performed at 12 sampling points along the coastal areas of Panggang Island, Karya Island, Pramuka Island, and Karang Congkak Semak Daun Island. The geographical coordinates of the sampling locations ranged between 106°36'31.70"E-106°36'35.10" E and 5°44'12.72"S-5°44'48.30" S (Figure 1). The sampling strategy included the collection of water for eDNA, chlorophyll-a, total suspended solids, water chemistry analysis, and plankton. Each site provided critical data for evaluating the water quality and ecological conditions.

For eDNA, chlorophyll-a, and total suspended solids, one liter of water was collected from each station and filtered using Whatman polytetrafluoroethylene (PTFE) membrane filters with a pore size of 0.45 µm. After filtration, the filter membranes were placed in sterile plastic bags and stored at -20°C until further analysis. This method effectively captured biological and particulate matter for subsequent laboratory tests. For water chemistry analysis, 0.5 liters of water from each site were collected in plastic bottles and kept on ice in an insulated ice box to preserve the integrity of the samples. This step was crucial for accurate chemical assessments later in the laboratory.

Plankton samples were collected by filtering 100 liters of seawater through a plankton net with a mesh size of 0.45 µm. The filtered plankton was then transferred to 50 mL Falcon tubes and preserved with a 10% Lugol iodine solution to ensure proper preservation for further analysis. This method enabled a detailed assessment of planktonic organisms in the water column.

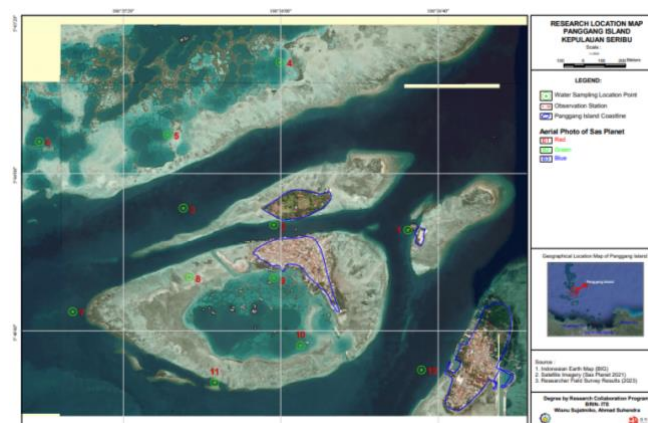


Figure 1. Sampling sites located in Kepulauan Seribu, Jakarta.

eDNA extraction. The collected filter membranes from 12 sampling sites in different seasons were placed into a 1.5 mL microtube for eDNA extraction. For the bacterial metagenome, the phenol-chloroform method as described by Sambrook et al (1989) was employed. For the plankton metagenome, tissue DNA isolation methods, also based on Sambrook et al. (1989), were used. The DNA extraction protocol adopted from Sambrook 1989 is summarized by Thomas, (2012) as follows: a. Cell Lysis Protein Digestion; add 400 μ L of extraction buffer (10mM Tris-HCL pH 8.0, 50 mM NaCl. 10 mM sodium dodecyl sulfate (SDS) and 20 μ L of 10 mg/mL Proteinase K to each tissue sample. Incubate @55°C for 2 hours or overnight. b. Sample Purification: add 400 μ L phenol to each sample and invert several times. Spin tubes at 13,000rpm for 5 min and pipette supernatant into a fresh 1.7 mL tube. c. DNA Precipitation: add 0.9 mL chilled 100% ethanol and 40 μ L of 3M sodium acetate pH 5.2 to each sample. Mix gently and place at 20°C for 60 min. Spin the sample at 13,000 rpm at 4°C for 30 min and then remove 100% ethanol and wash with 70% ethanol. Spin at 13,000 rpm at 4°C for 10 min. Remove ethanol from a tube and let the pellet dry. d. DNA Re-Hydration: Re-suspend in 50 μ L TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA); Allow to sit for 1 hour at room temperature before freezing. To estimate DNA concentration using agarose gel electrophoresis: agarose Gel PCR Product with primer 18S forward and 18S reverse with KOD One™ PCR Master Mix -Blue-(Toyobo, KMM-201) and primer V3V4 using KOD-Multi & Epi™ (Toyobo, KME-101), DNA concentration and purity ratios (A260/A280 values) were measured using a nano-drop. NGS quality control matrices use GC content and estimation of confidence from the Phred-Encoded Quality Score. Following extraction, DNA from the 12 samples was pooled by season, resulting in two composite samples for each metagenome: the dry and rainy seasons. In total, two bacterial metagenome samples and two plankton metagenome samples were analyzed.

Table 1

Primer pairs and PCR procedures used in this study

Primer	Sequence	Procedures	Reference
TAReuk454FWD1	5'-CCA GCA SCY GCG GTA ATT CC-3'	5 min at 94°C, 35 cycles: 1 min at 94°C, 1 min at 53°C, 1 min at 72°C, and 5 min at 72°C	Stoeck et al 2010
TAReukREV3	5'-ACT TTC GTT CTT GAT YRA TGA-3'		
341F	5'-CCT AYG GGR BGC ASC AG-3'	30 sec at 98°C, 30 cycles: 5 sec at 98°C, 20 sec at 56°C, 20 sec at 72°C, and 5 min at 72°C	Michelsen et al 2014
806R	5'-GGA CTA CNN GGG TAT CTA AT-3		

Next-generation sequencing and data analysis. Extracted eDNA samples were amplified with the primer listed in Table 1. gDNA samples were amplified with target-specific primers (18S Forward and 18S Reverse) and (16SV3-V4). Library preparation was conducted using the amplified PCR product. The library was subsequently sequenced on the MGI-DNBSEQ-G400 platform to generate paired-end raw reads. Adapter and PCR primer sequences from the paired-end read were then removed using Cutadapt (Bellemain et al 2010; Martin 2011). To correct sequencing errors, and remove low-quality sequences, and chimera errors, DADA2 was used (Martin, 2011; Callahan et al 2016). The resulting ASVs (Amplicon sequence variant) data was used for taxonomic classification against PC2_18S-pr2_version_5.0.0_SSU and SILVA (silva_nr99_v138.1). The quality control processes in NGS were also conducted to verify that the data quality met the required standards for downstream analysis. Downstream analysis and visualizations were performed using packages in RStudio (R version 4.2.3)(R Core Team 2021), Krona Tools (Ondov et al 2011), and PICRUST2 (Douglas et al 2020). Statistical analysis of the taxonomy assignment and abundance of each sample was performed using the resulting representative sequences from DADA2. The bacterial and plankton richness and diversity were analyzed using the Shannon Index, the Simpson Index, and the InvSimpson value.

Water quality analysis. In this study, both physical and chemical water quality parameters were assessed. The physical parameters included current velocity, water clarity, temperature, salinity, total suspended solids, pH, BOD5 (Biochemical Oxygen Demand), and dissolved oxygen. The chemical parameters measured were NO₃, PO₄, NH₃, oil content, and chlorophyll-a. These indicators were chosen to provide a comprehensive overview of water quality conditions. Physical parameters such as temperature, salinity, pH, total suspended solids, and dissolved oxygen were measured using the Horiba U-50 Multi-parameter water quality checker. This instrument allows for precise and real-time measurement of these indicators, ensuring consistent results.

For the analysis of chemical parameters, NO₃, PO₄, and NH₃ concentrations were determined using HACH Test Kits and Strips. The readings were taken using a HACH Multiparameter Portable Colorimeter DR900, following the procedures outlined in the manufacturer's guidelines. BOD5 was measured following the method described by (Mizwar & Surapati 2020), which ensures accuracy in measuring biochemical oxygen demand over a five-day period. Chlorophyll-a levels were determined by dissolving filter paper in 90% acetone. The sample was centrifuged at 5000 rpm for 30 minutes, and the supernatant was analyzed using a Trilogy fluorometer 7200-046. This method provides a reliable measure of chlorophyll-a concentration, reflecting the productivity of aquatic systems. Finally, the oil content was analyzed using a distillation method as described by Ariani et al (2016), ensuring precise quantification of hydrocarbon contamination in the water samples. Statistical test for differences in water quality between the rainy and dry seasons using the Tukey method.

Result

DNA amplification test. The conventional PCR result showed that 16S and 18S rDNA were successfully amplified from the eDNA samples (Figure 2). Gel electrophoresis revealed a 446 bp band for 16S rDNA and 400 bp band for 18S rDNA. The amplified bands were distinct and relatively thick, with minimal smear, indicating high PCR efficiency and successful amplification of eDNA at the target locations by the primers. These results provide clear evidence that the eDNA was successfully amplified, allowing for accurate downstream analysis, and indicate that the samples contain sufficient high-quality eDNA for NGS studies. Additionally, the uniformity and intensity of the bands across replicates further underscore the reproducibility and reliability of the amplification process in this study.

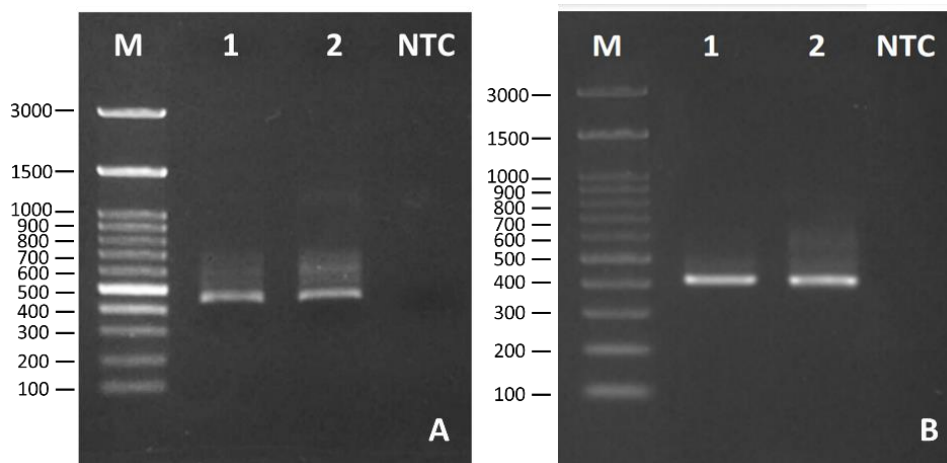


Figure 2. Amplification test result of the extracted eDNA. A: amplification test using 16S rDNA primer; B: amplification test using 18S rDNA primer. 1: rainy season; 2: dry season. M: marker; NTC: no template control.

NGS quality control metrics. The NGS result indicated that the final read counts across samples ranged from 10.996 to 38.063, with an average nucleotide length of approximately 300 bp. The guanine-cytosine (GC) content of the sequences varied between 45% and 51%, reflecting a moderate GC distribution across the data (Table 2). Additionally, the Phred quality scores, a critical metric for assessing sequencing accuracy, consistently exceeded 20 for all samples, demonstrating a high level of confidence in the base calls and supporting their suitability for downstream bioinformatic analyses.

Table 2
Summary of quality control metrics for NGS data across samples

Sample	Final Reads	Nucleotide Length	GC %	Phred Score
Rainy Season Bacteria	13.089	300	51%	35.45-25.17
Dry Season Bacteria	10.996	300	51%	33.81-27.11
Rainy Season Plankton	38.063	300	45%	35.62-26.93
Dry Season Plankton	27.773	300	45%	36.96-20.96

Class-level abundance of bacteria and plankton. In both the rainy and dry seasons, Alphaproteobacteria dominated the environment, comprising 58.48% and 53.8% of the microbial community, respectively, followed by Cyanobacteria as the second most abundant group (Figure 3). In the rainy season, the third most abundant group was Bacterioidia (13.08%), while in the dry season was Acidimicrobiia (9.88%). In the dry season, the percentage of cyanobacteria (34.22%) was higher than that of the rainy season (18.59%). Analysis using the 18S gene revealed that Arthropoda (40.25%) dominated the water during the rainy season, followed by the Echinodermata group (22.79%). In the dry season, microalgae Mamiellophyceae (37.22%) dominated the environment followed by Syndiniales (16.31%) as the second most abundant group. The Arthropoda, which is the most abundant group in the rainy season was the third most abundant group (9.39%) in the dry season. Based on the analysis, zooplankton were identified as the most and the second most abundant groups during the rainy season, while phytoplankton dominated during the dry season.

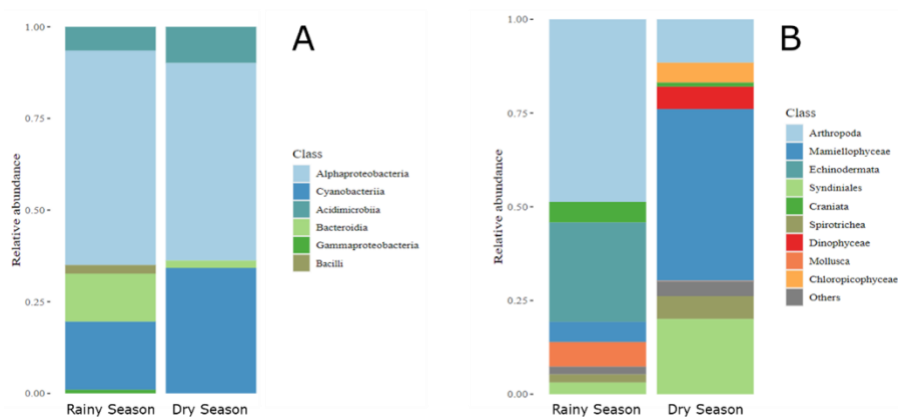


Figure 3. Class-level abundance of bacteria (A) and plankton (B) in the samples.

Family-level abundance of bacteria and plankton. During the rainy season, the bacterial community was dominated by Rhodobacteraceae, which emerged as the most abundant group, followed by Clade 1 bacteria and Cyanobiaceae as the second and third most abundant groups, respectively (Figure 4). In contrast, during the dry season, Cyanobiaceae became the dominant group, with Clade 1 bacteria maintaining its position as the second most abundant group, and Rhodobacteraceae decreasing to third place in abundance. The plankton community displayed a similar seasonal variation. In the rainy season, Maxillopoda was the most dominant group (39.82%), with Echinodermata being the second most abundant (22.79%). However, in the dry season, Bathycoccaceae became

the dominant plankton group (21.45%), followed closely by Mamiellaceae (15.78%). These seasonal shifts in both bacterial and plankton populations suggest that environmental factors, particularly those associated with seasonal changes, play a crucial role in shaping the microbial and planktonic ecosystems in the ocean.

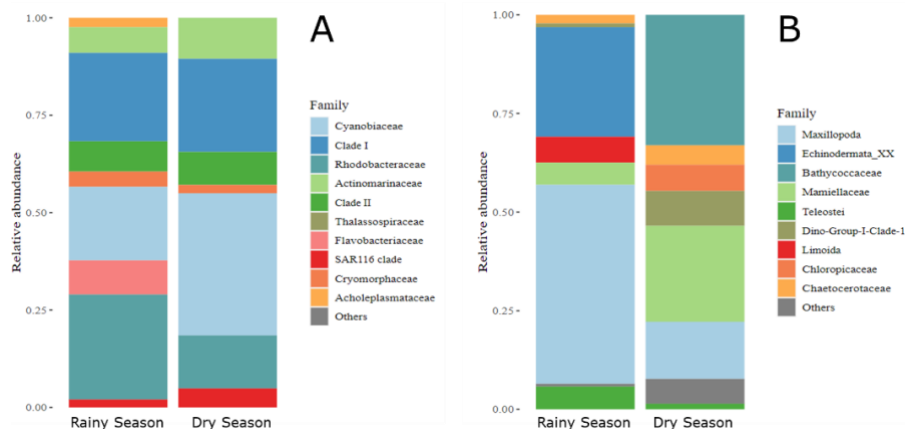


Figure 4. Family-level abundance of bacteria (A) and plankton (B) in the samples.

The genus-level abundance of bacteria and plankton. During the rainy season, HIMB11 was the dominant microbial group, accounting for 26.51% of the total, followed by Clade Ia at 22.33% and *Synechococcus* CC9902 at 15.31% (Figure 5). In contrast, during the dry season, *Synechococcus* CC9902 became the most dominant, comprising 27.07%, followed by Clade Ia at 22.40% and HIMB11 at 12.76%. In terms of planktonic abundance, no single group showed significant dominance during the rainy season. *Dioithona* was the most abundant planktonic group, representing 8.86%, closely followed by *Bestiolina* (8.75%) and *Undinula* (8.27%). In the dry season, *Ostreococcus* dominated with 21.44%, followed by *Micromonas* at 15.78%, making it the second most abundant group. Dinoflagellates, which are often responsible for harmful algal blooms, did not dominate the water in either season. During the rainy season, dinoflagellates only accounted for 1.13% of the total planktonic community, while in the dry season, they comprised 5.74%. This indicates a relatively low presence of harmful algal bloom-forming dinoflagellates across both periods.

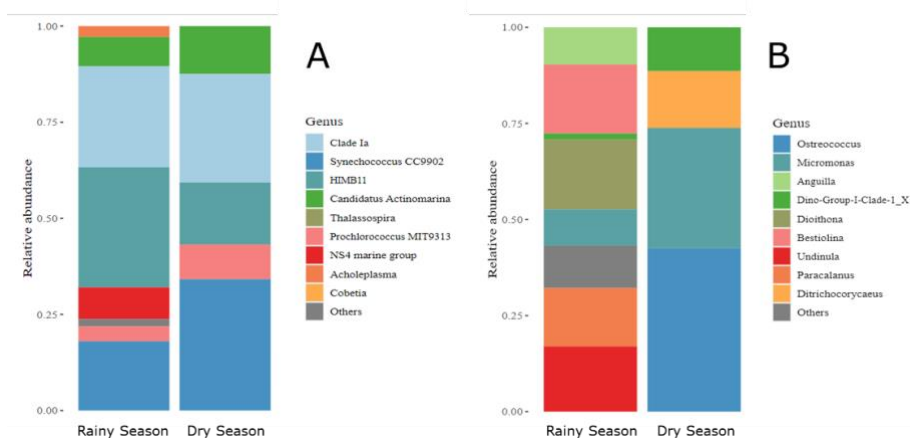


Figure 5. Genus-level abundance of bacteria (A) and plankton (B) in the samples.

Diversity analysis. The bacterial richness observed during the rainy season was higher, with a total of 20 groups, compared to the dry season, which recorded only 14 groups (Table 3). The Shannon index for bacteria in both seasons was approximately 2, with the rainy season exhibiting a higher value. This indicates that the bacterial community is fairly diverse, with several groups present and some degree of evenness in their distribution. Additionally, the Simpson index for bacteria in the rainy and dry seasons was around 0.8, with the rainy season showing a higher value. This suggests that while some diversity

exists, the community is somewhat dominated by certain groups. The InvSimpson values for bacteria were 7.7017 in the rainy season and 6.9658 in the dry season, indicating that the bacterial community is not dominated by just one or a few species but instead maintains a rich and even distribution across different groups.

In contrast, the plankton richness was higher during the rainy season, with a total of 44 groups, compared to 68 groups recorded in the dry season. The Shannon index for plankton was approximately 2 in the rainy season, while it exceeded 3 in the dry season. This suggests that the plankton community was fairly diverse in the rainy season, whereas in the dry season, it exhibited a more balanced and diverse composition. The Simpson index for plankton in both seasons was around 0.9, indicating that certain groups dominated the environment. The InvSimpson values for plankton were around 11 in the rainy season and 19 in the dry season, suggesting that the community in the dry season was more diverse compared to the rainy season.

Table 3

Diversity analysis of bacteria and plankton during the rainy season and dry season

<i>Sample</i>	<i>Observed</i>	<i>Shannon</i>	<i>Simpson</i>	<i>InvSimpson</i>
Rainy Season Bacteria	20	2.3594	0.8702	7.7017
Dry Season Bacteria	14	2.1938	0.8564	6.9658
Rainy Season Plankton	44	2.8997	0.9091	11.0047
Dry Season Plankton	68	3.5059	0.9478	19.1714

Water quality parameters. In this study, the ocean water quality parameters varied significantly between the dry and rainy seasons (Table 4). During the dry season, current velocity, temperature, salinity, dissolved oxygen, and ammonia (NH₃) were notably higher than in the rainy season, suggesting enhanced water movement and aeration. Conversely, the rainy season exhibited significantly elevated total suspended solids (TSS) and pH levels, likely due to increased runoff and freshwater input. Other parameters, including water clarity, biological oxygen demand (BOD₅), nitrate (NO₃), phosphate (PO₄), oil content, number of plankton, and chlorophyll-a, showed no significant differences between the two seasons, indicating stable concentrations regardless of seasonal changes.

Table 4

Water quality parameters on the sampling site

<i>Parameters</i>	<i>Rainy season</i>	<i>Dry season</i>	<i>Standard</i>	<i>Reference</i>
Current velocity (cm/s)	15.27±4.87 ^a	30.78±7.81 ^b	20-30	Warnadi et al 2018
Water clarity (m)	7.04±2.97 ^a	5.71±2.04 ^a	>5	MERI 2004
Temperature (°C)	29.94±0.23 ^a	30.29±0.35 ^b	Natural	
Salinity (ppt)	28.85±0.16 ^a	29.36±0.33 ^b	33-37	ANZECC 2000
Total suspended solid (mgL ⁻¹)	16.82±2.08 ^b	13.33±4.21 ^a	<20	MEF 2021
pH	8.1±0.07 ^b	7.63±0.03 ^a	6-9	ANZECC 2000
BOD ₅ (mgL ⁻¹)	1.58±0.45 ^a	1.4±0.34 ^a	2	Simon et al 2011
Dissolved oxygen (mgL ⁻¹)	4.84±0.41 ^a	5.81±0.98 ^b	>5	Lawson 1995
NO ₃ (mgL ⁻¹)	3.7±1.56 ^a	2.91±1.04 ^a	<10	MEF 2021
PO ₄ (mgL ⁻¹)	0.15±0.07 ^a	0.2±0.14 ^a	<0.2	MEF 2021
NH ₃ (mgL ⁻¹)	0.06±0.01 ^a	0.08±0.02 ^b	<0.3	MEF 2021
Oil contents (mgL ⁻¹)	23.17±14.8 ^a	26.83±16.33 ^a	<1	Marbun et al 2009
Plankton (IndividuL ⁻¹)	1,189.92±2,584.15 ^a	125.92±38.37 ^a	Not bloom	MEF 2021
Chlorophyll-a (µgL ⁻¹)	1.56±1.5 ^a	0.93±0.36 ^a	<1	ANZECC 2000

Different letters indicate significant differences (p<0.05) based on the Tukey method.

Discussion. In metagenomic analysis, many genes are utilized to explore the diversity of microbial communities. The 18S rRNA gene has emerged as a prominent marker in DNA metabarcoding studies of plankton because of its ability to effectively identify a broad spectrum of eukaryotic microorganisms despite its limitations in achieving resolution at the species level (Bukin et al 2023; Romadhona et al 2024). Similarly, the 16S rRNA gene is frequently employed for the metabarcoding of bacterial species, allowing researchers to detect and quantify bacterial diversity in complex environments such as soil or microbiomes (Zemb et al 2020). Before performing next-generation sequencing (NGS), it is crucial to confirm the sample's amplifiability using conventional PCR. This step ensures that the primers are well-suited to the target regions and that sufficient DNA is present for successful amplification (Malkawi et al 2010). In this study, all samples were amplified using both 16S rRNA and 18S rRNA primer pairs, confirming that the DNA concentration was adequate for NGS analysis. This validation step is critical in ensuring the quality and reliability of the sequencing results.

The quality control metrics for the NGS data in this study demonstrated that the Phred scores were consistently above 20, widely recognized as a threshold for high-quality base calling in sequencing. A Phred score of 20 corresponds to an estimated base calling error rate of only 1% (Nielsen et al 2011; Shi et al 2016), ensuring the reliability of the data used for downstream analysis (Sathyanarayanan et al 2018). Furthermore, the GC content of the sequences ranged from 45% to 51%, which falls within the optimal range of 40% to 60% typically required for NGS data. Maintaining this range is crucial to minimize bias and ensure the accuracy of the sequencing results and subsequent analyses (Chen et al 2013). However, the nucleotide lengths observed in this study were shorter than expected, as shown in Figure 2. This reduction in length can be attributed to computational trimming processes that remove low-quality bases, which often results in shorter but higher-quality read lengths (Singh et al 2021). These combined factors highlight the overall quality and robustness of the data, despite the observed reduction in read lengths.

The plankton community exhibited a seasonal shift, with zooplankton dominating during the rainy season and phytoplankton taking over in the dry season. This seasonal variation aligns with studies that suggest zooplankton thrive in nutrient-rich waters typically found after rainfall, whereas phytoplankton, such as microalgae, dominate in more stable, nutrient-limited conditions during the dry season (Hoover et al 2006; Bilbao et al 2023). No significant difference in nutrient levels was observed between seasons, except for ammonia. The dominance of microalgae in the dry season may be influenced by increased sea surface temperatures and salinity (Oyeku & Mandal 2021).

Arthropoda and Mamiellophyceae were the dominant class in the rainy and dry seasons, respectively. Arthropods, including crustaceans like copepods and amphipods, play crucial roles in marine ecosystems, contributing to aquaculture as feed sources (e.g., copepods for fish larvae) and ecosystem stability (Støttrup 2000; Ritter & Bourne 2024). Maxillopoda, a subclass of Arthropoda, exhibited increased abundance during the rainy season, similar to findings reported by Muhtadi et al (2022). Mamiellophyceae, unicellular green algae, dominated in the dry season, significantly influencing primary production and nutrient cycling (Tragin & Vaultot 2019). No dominance of Dinoflagellata, typically responsible for harmful algal blooms, was observed in either season.

NGS analysis for bacteria showed Alphaproteobacteria including Rhodobacteriaceae was dominant in the ocean in both the rainy and dry seasons. Domination of Alphaproteobacteria was also reported by Biers et al (2009). Domination in both seasons may occur because many clades adapted to various oceanic regions, emphasizing their adaptability to different environmental conditions (Hördt et al 2020). Alphaproteobacteria have a significant role and influence ecosystem stability by participating in organic matter degradation and enhancing nutrient cycling in marine environments (Kunihiro et al 2011). The second most abundant group in both seasons was Cyanobacteria, especially in the dry season, while the percentage of Cyanobacteria was higher compared with the rainy season. Increased nutrient levels, such as nitrogen from human activities like agriculture and urban runoff, and increased water temperature promote cyanobacterial growth, leading to blooms (Gophen 2021; Wang et al 2021). This is consistent with the findings of this study, which show that NH_3 in the dry season was significantly higher than rainy season. The genus-

level NGS analysis confirmed the absence of pathogenic bacterial genera associated with ice-ice disease in the studied environment. Known bacteria genera that contribute to ice-ice disease include *Vibrio*, *Alteromonas*, *Bacillus*, and *Pseudomonas* (Tuhumury et al 2024). The findings suggest that the waters surrounding the Kepulauan Seribu are suitable for seaweed cultivation, despite seasonal variations in bacterial diversity and dominance.

The water quality in the Kepulauan Seribu region, including parameters such as total chlorophyll and oil content, was found to be slightly above standard limits, indicating the presence of anthropogenic pollution. Despite these environmental concerns, there have been reports of successful seaweed in the area. For instance, Verdian et al (2020) reported that Integrated Multi-Trophic Aquaculture (IMTA), involving whiteleg shrimp (*Penaeus vannamei* Boone, 1931), rabbitfish (*Siganus doliatus* Guérin-Méneville, 1829-38), and *Kappaphycus alvarezii* (Doty) Doty ex P.C.Silva, 1996 achieved promising results. Furthermore, Warnadi et al (2018) highlighted that the Kepulauan Seribu waters remain suitable for seaweed cultivation, despite the medium-level pollution detected in the area. Aris & Labenua (2020) concluded that the waters on Panggang Island, DKI Jakarta are very suitable for cultivating *Kappaphycus alvarezii* seaweed, both in the rainy and dry seasons. These findings suggest that while anthropogenic impacts are evident, aquaculture practices, continue to be viable in this region, contributing to both environmental sustainability and economic development.

Conclusion. NGS analysis provided valuable insights into the bacterial and plankton diversity in the Kepulauan Seribu, identifying Alphaproteobacteria as the dominant bacterial group in both the rainy and dry seasons. The ecosystem exhibited seasonal shifts, with zooplankton dominating during the rainy season and phytoplankton prevailing in the dry season. Importantly, no harmful algal blooms or pathogenic bacteria were detected, reinforcing the suitability of the area for marine aquaculture. Despite the presence of some anthropogenic influences, the overall microbial and planktonic composition suggests that the waters around the Kepulauan Seribu remain conducive to seaweed culture activities. Further research is required to identify the most suitable seaweed commodities for cultivation in this region.

Conflict of interest. The authors declare that there are no conflicts of interest regarding the authorship or publication of this paper.

References

- ANZECC, 2000 Australia and New Zealand guidelines for fresh and marine water quality. Vol. 1. Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand.
- Ariani F., Effendi H., Suprihatin, 2016 Water and sediment oil content spread in Dumai coastal waters, Riau Province, Indonesia. *Egyptian Journal of Aquatic Research* 42(4):411–416.
- Aris M., Labenua R., 2020 Evaluation of land suitability of *Kappaphycus alvarezii* cultivation in the dry and rainy season. *IOP Conference Series: Earth and Environmental Science* 584(1).
- Aziz, Achmad M., Indarjo A., Salim G., Rozi., 2022 Identification of bacteria in seaweed (*Kapappphycus alvarezii*) affected by the ice-ice disease in Amal and Tanjung Tarakan waters, Indonesia. *AAFL Bioflu* 15(6):3061–3068.
- Bellemain E., Carlsen T., Brochmann C., Coissac E., Taberlet P., Kausrud H., 2010 ITS as an environmental DNA barcode for fungi: an insilico approach reveals potential PCR biases. *BMC microbiology* 10:1-9.
- Biers E. J., Sun S., Howard E. C., 2009 Prokaryotic genomes and diversity in surface ocean waters: Interrogating the global ocean sampling metagenome. *Applied and Environmental Microbiology* 75(7):2221–2229.
- Bilbao J., Pavloudi C., Blanco-Rayón E., Franco J., Madariaga I., Seoane S., 2023 Phytoplankton community composition in relation to environmental variability in the

- Urdaibai estuary (SE Bay of Biscay): Microscopy and eDNA metabarcoding. *Marine Environmental Research* 191:106175.
- Bukin Y. S., Mikhailov I. S., Petrova D. P., Galachyants Y. P., Zakharova Y. R., Likhoshway Y. V., 2023 The effect of metabarcoding 18S rRNA region choice on diversity of microeukaryotes including phytoplankton. *World Journal of Microbiology and Biotechnology* 39(9):229.
- Chen Y. C., Liu T., Yu C. H., Chiang T. Y., Hwang C. C., 2013 Effects of GC bias in next-generation-sequencing data on de novo genome assembly. *PLoS ONE* 8(4):e62856.
- Callahan B. J., McMurdie P. J., Rosen M. J., Han A. W., Johnson A. J. A., Holmes S. P., 2016 DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods* 13:581-583.
- Douglas G. M., Maffei V. J., Zaneveld J. R., Yurgel S. N., Brown J. R., Taylor C. M., Huttenhower C., Morgan G. I. L., 2020 PICRUSt2 for prediction of metagenome functions. *Nature Biotechnology* 38:685-688.
- Gophen M., 2021 Climate change-enhanced cyanobacteria domination in lake Kinneret: A retrospective overview. *Water (Switzerland)* 13(2):163.
- Hastuti, Muhidu A., Agus Mokodompit E., Program D., 2023 indonesia's marine economic potential as a maritime country. *International Journal of Science* 813-825.
- Hendrayanti D., Prihantini N. B., Ningsih F., Maulana F., 2023 Next generation sequencing (ngs) for cyanobacterial study in Agung and Sunter Barat Lakes, North Jakarta, Indonesia. *Biodiversitas* 24(2):1117-1124.
- Hoover R. S., Hoover D., Miller M., Landry M. R., DeCarlo E. H., Mackenzie F. T., 2006 Zooplankton response to storm runoff in a tropical estuary: Bottom-up and top-down controls. *Marine Ecology Progress Series* 318:187-201.
- Hördt A., López M. G., Meier-Kolthoff J. P., Schleuning M., Weinhold L. M., Tindall B. J., Gronow S., Kyrpides N. C., Woyke T., Göker M., 2020 Analysis of 1,000+ type-strain genomes substantially improves taxonomic classification of *Alphaproteobacteria*. *Frontiers in Microbiology* 11:468.
- Kunihiro T., Takasu H., Miyazaki T., Uramoto Y., Kinoshita K., Yodnarasri S., Hama D., Wada M., Kogure K., Ohwada K., Tsutsumi H., 2011 Increase in *Alphaproteobacteria* in association with a polychaete, *Capitella* sp. I, in the organically enriched sediment. *ISME Journal* 5(11):1818-1831.
- Lawson T. B., 1995 *Fundamentals of aquacultural engineering*. Chapman & Hall.
- Malashenkov D. V., Dashkova V., Zhakupova K., Vorobjev I. A., Barteneva N. S., 2021 Comparative analysis of freshwater phytoplankton communities in two lakes of Burabay National Park using morphological and molecular approaches. *Scientific Reports* 11:16130.
- Malkawi H. I., Al-Omari M. N., Ashounah J., Ma Z 2010 Culture-dependent and culture-independent approaches to study the bacterial and archaeal diversity from Jordanian hot springs Culture-dependent and culture-independent approaches to study the bacterial and archaeal diversity from Jordanian hot springs the microbial diversity was investigated at five major hot springs in. *Article in African Journal of Microbiology Research* 4(10):923-932.
- Marbun Y. R., Muntalif B. S., Muin M., 2009 Spreading analysis of phenol, oil and grease of produced water from oil and gas field and their impacts to offshore environment. *Jurnal Teknik Lingkungan* 15(1):18-28.
- Martin M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet. journal* 17(1):10-12.
- MEF (The Ministry of Environment and Forestry), 2021 [Government Regulation of the Republic of Indonesia No. 22 of 2021 on the Implementation of Environmental Protection and Management]. [In Indonesian]
- MERI (Decree of The State Minister of Environment of the Republic of Indonesia), [Number 179 of 2024 regarding amendment on Decree of The State Minister of Environment Number 51 of 2024 Regarding Standard Quality of Seawater]. [In Indonesian]
- Michelsen C. F., Pedas P., Glaring M. A., Schjoerring J. K., Stougaard P., 2014 Bacterial diversity in Greenlandic soils as affected by potato cropping and inorganic versus organic fertilization. *Polar Biology* 37(1):61-71.

- Mizwar A., Surapati H. U., 2020 Biological oxygen demand (BOD₅) as bio indicator of phytoplankton diversity index in the mangrove area of Kintap Estuary - South Kalimantan. IOP Conference Series: Earth and Environmental Science 448(1):012126.
- MMAF (The Ministry of Marine Affairs and Fisheries), 2024 *Analysis of Key Performance Indicators in the Marine and Fisheries Sector for the Period 2019-2023* (Vol. 2).
- Muhtadi A., Yulianda F., Boer M. F., Krisanti M., 2022 Spatial distribution management of Crustacea (Decapoda) based on conservation in tropical tidal lake. Biodiversitas 23(8):4064-4072.
- Nafea A. M., Wang Y., Wang D., Salama A. M., Aziz M. A., Xu S., Tong Y., 2023 Application of next-generation sequencing to identify different pathogens. *Frontiers in Microbiology* vol. 14:1329330.
- Nielsen R., Paul J. S., Albrechtsen A., Song Y. S., 2011 Genotype and SNP calling from next-generation sequencing data. *Nature Reviews Genetics* 12(6):443-451.
- Ondov B. D., Bergman N. H., Phillippy A. M., 2011 Interactive metagenomic visualization in a Web browser. *BMC Bioinformatics* 12:385.
- Oyeku O. G., Mandal S. K., 2021 Historical occurrences of marine microalgal blooms in Indian peninsula: Probable causes and implications. *Oceanologia* 63(1):51-70.
- Ritter C. J., Bourne D. G., 2024 Marine amphipods as integral members of global ocean ecosystems. *Journal of Experimental Marine Biology and Ecology* 572:151985.
- R Core Team, 2021 R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Romadhona E. I., Megawati N., Widantara H., Sutanti S., Rahman Ardiansyah A., Mariya Dewi K., Fitri Larassagita A., Firman A., Risky M., Rahmat R., Ayub A., 2024 Characterization of CO1 and 18S rDNA gene from the domesticated Indonesian native ornamental shrimp *caridina boehmei*. *Jurnal Akuakultur Indonesia* 23(2):147-156.
- Sambrook J., Fritsch E., Maniatis T., 1989 *Molecular cloning: a laboratory manual* (2nd ed.). Cold Spring Harbor Laboratory Press.
- Sathyanarayanan A., Manda S., Poojary M., Nagaraj S. H., 2018 Exome sequencing data analysis. *Encyclopedia of Bioinformatics and Computational Biology: ABC of Bioinformatics* 1-3:164-175.
- Shi H., Li W., Xu, X., 2016 Learning the comparing and converting method of sequence phred quality score. 6th International Conference on Management, Education, Information and Control 260-263.
- Simon F. X., Penru Y., Guastalli A. R., Llorens J., Baig S., 2011 Improvement of the analysis of the biochemical oxygen demand (BOD) of Mediterranean seawater by seeding control. *Talanta* 85(1):527-532.
- Singh A. K., Olsen M. F., Lavik L. A. S., Vold T., Drabløs F., Sjørusen W., 2021 Detecting copy number variation in next generation sequencing data from diagnostic gene panels. *BMC Medical Genomics* 14(1):214.
- Stoeck T., Bass D., Nebel M., Christen R., Jones M. D. M., Breiner H. W., Richards T. A., 2010 Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. *Molecular Ecology*, 19(SUPPL. 1):21-31.
- Støttrup J. G., 2000 The elusive copepods: their production and suitability in marine aquaculture. *Aquaculture Research* 31:703-711.
- Suhendra A., Karnaningroem N., Nugroho R., Sujatmiko W., 2024 The impact of oil and fat pollutants on the aquatic environment around Panggang Island, Kepulauan Seribu, on the lifespan of seaweed (*Kappaphycus alvarezii*). *Jurnal Serambi Engineering* 9(1):8237-8245.
- Tragin M., Vaulot D., 2019 Novel diversity within marine *Mamiellophyceae* (*Chlorophyta*) unveiled by metabarcoding. *Scientific Reports* 9(1):5190.
- Tran N., Rodriguez U. P., Chan C. Y., Phillips M. J., Mohan C. V., Henriksson P. J. G., Koeshendrajana S., Suri S., Hall S., 2017 Indonesian aquaculture futures: An analysis of fish supply and demand in Indonesia to 2030 and role of aquaculture using the AsiaFish model. *Marine Policy* 79:25-32.
- Thomas J., 2012. Population genetic of the Red Rock Lobster, *Jasus edwardsii*. Thesis, Victoria University of Wellington, Wellington, New Zealand.

- Tuhumury N. C., Sahetapy J. M. F., Matakupan J., 2024 Isolation and identification of bacterial pathogens causing ice-ice disease in *Eucheuma cottonii* seaweed at Seira Island Waters, Tanimbar Islands District, Maluku, Indonesia. *Biodiversitas* 25(3):964–970.
- Verdian A. H., Effendi I., Budidardi T., Diatin I., 2020 Production performance improvement of white shrimp (*Litopenaeus vannamei*) culture with integrated multi trophic aquaculture system in Seribu Islands, Jakarta, Indonesia. *Iranian Journal of Fisheries Sciences* 19(3):1415–1427.
- Wang Z., Akbar S., Sun Y., Gu L., Zhang L., Lyu K., Huang Y., Yang Z., 2021 Cyanobacterial dominance and succession: Factors, mechanisms, predictions, and managements. *Journal of Environmental Management* 297:113281.
- Warnadi S., Setyaningsih A. I., Kasih W. A., 2018 Water quality and it's effect on seaweed cultivation in Pari Island, Kepulauan Seribu DKI Jakarta. *IOP Conference Series: Earth and Environmental Science* 145(1).
- Wijianto W., Effendi I., Diatin I., Budidardi T., Hadiroseyani Y., 2024 [Factors affecting seaweed cultivation production in pekalongan regency, Central Java Province. *Jurnal Perikanan Unram* 14(3):1096–1101]. [In Indonesian]
- Zemb O., Achard C. S., Hamelin J., De Almeida M. L., Gabinaud B., Cauquil L., Verschuren L. M. G., Godon J. J., 2020 Absolute quantitation of microbes using 16S rRNA gene metabarcoding: A rapid normalization of relative abundances by quantitative PCR targeting a 16S rRNA gene spike-in standard. *MicrobiologyOpen* 9(3):e977.
- Zhu Y., Wang Z., Song L., Christakos G., Agusti S., Duarte C., Wu J., 2022 Dynamics of phytoplankton community in relation to seasonal variation and *Laminaria japonica* culturing in coastal area. *Estuarine, Coastal and Shelf Science* 277:108063.

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